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Erratum

In the October, 1999, article by Schaefer et al., "Live cell fluorescence imaging of T cell MEKK2: redistribution and activation in response to antigen stimulation of the T cell receptor," a cell line was misidentified. We would therefore like to publish a correction.

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The cell line identified in the abovementioned publication as D10/MEKK2-GFP was actually D10/PKCtheta-GFP. This error occurred as a result of the accidental switching of these two cell lines during a time period in which several GFP fusion proteins were concomitantly introduced into the D10 T cell clone. The switching of these cell lines was not detected for several reasons: (1) PKCtheta-GFP and MEKK2-GFP have precisely the same SDS-PAGE mobility; (2) because good quality anti-MEKK2 antibodies for Western blotting were not available at the time that the original experiments were performed, the fusion protein was detected in Western blotting experiments with an anti-GFP antibody, a procedure that would have detected either MEKK2-GFP or PKCtheta-GFP; (3) MEKK2 and PKCtheta are both serine-threonine kinases, and the fusion protein thus had the expected biochemical activity in kinase assays; and (4) the imaging data of the GFP fusion protein supported the results obtained by antibody staining of endogenous MEKK2.

PCR and DNA sequence analysis have now confirmed that only the D10/MEKK2-GFP cell line was misidentified. A new cell line, D10/MEKK2-YFP, was established for the purpose of reproducing our original data. Unfortunately, we have discovered that although the data showing antigen-stimulated redistribution of endogenous MEKK2 are consistent and reproducible (original Figure 1A, and new Figure 1, below), the MEKK2-YFP fusion protein does not redistribute to a detectable extent in response to antigen stimulation, nor is it biochemically activated by antigen stimulation (Figures 1B-1D, 2A and 2B, and 3A). The most likely explanation for this observation is that an upstream signal or adaptor molecule is limiting, and that, in the presence of overexpressed MEKK2-YFP, only a small percentage of the total MEKK2-YFP becomes activated and redistributed in response to antigen stimulation. The resulting signal-to-noise ratio is thus too low to allow detection of activation or redistribution.

The data in the remainder of the paper (Figures 3B–3D, 4A–4D, and 5A and 5B), which provide evidence that MEKK2 is involved in activation of the T cell ERK1/2 and p38 MAP kinase pathways, that MEKK2 is not involved in TCR-regulated IL-4 production, that MEKK2 signaling contributes to TCR-regulated adhesion to antigen-presenting cells, and that PI-3 kinase is an upstream regulator of MEKK2, remain essentially unchanged when repeated with the newly established D10/MEKK2-YFP cell line. The one minor exception is that the enhancement of T cell adhesion seen at high antigen concentration with the (misidentified) D10/MEKK2-GFP cell line is not observed with the D10/MEKK2-YFP cell line (Figure 3C; the D10/MEKK2-YFP cell line behaves like the D10 control). Thus, although experiments using the MEKK2-YFP fusion protein provide no information regarding the kinetics or extent of activation and redistribution of MEKK2 in response to antigen stimulation, the major biological conclusions of the paper remain valid. We apologize for any effort and loss of time that our incorrect data may have caused other investigators.